

NASA TECH BRIEF

Goddard Space Flight Center



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Improved Methods for Counting Bacteria in Physiological Fluids

The problem:

Most methods used for counting bacteria in physiological fluids (e.g., blood and urine) rely on the growth of the bacteria and subsequent visual counting of colonies. Normally, a fluid sample is mixed with a nutrient medium and allowed to grow. Culture growth may take up to several days, delaying treatment.

The solution:

A method for detecting bacterial population that does not depend on growth is based on the detection of adenosine triphosphate (ATP), a chemical present in all living matter. The amount of ATP in a sample, after chemically removing all nonbacterial ATP, is directly related to the bacterial population. This method improves on the method described in the NASA Tech Brief B71-10051 (GSC-11092) in two ways. The sensitivity is improved by a concentration step, thereby detecting the presence of lower levels of bacteria. The specificity is improved by lowering the pH of the solution to 4.0, eliminating the measurement of nonbacterial ATP. Bacterial ATP can be measured even in the presence of as much as 10^6 leucocytes per ml.

How it's done:

Infected fluids, for example urine, include nonbacterial cells, such as epithelial cells, erythrocytes, leucocytes, and renal cells. All of these contain ATP, as does bacteria. Soluble ATP also is excreted in the urine. Therefore, an important step in the bacterial ATP detection process is to first destroy the ATP associated with the nonbacterial cells and soluble ATP and leave only bacterial ATP.

The nonbacterial cells are ruptured by a nonionic detergent such as octyl phenoxy polyethoxyethanol which does not affect bacterial cells. This process requires approximately ten minutes. The bacteria are concentrated by centrifugation. Next, the soluble ATP is destroyed by adding apyrase, an enzyme that hydrolyzes the freed and soluble ATP. The pH of the solution is lowered by the addition of malate buffer pH 3.75. This desorbs the ATP bound to particulates and allows the apyrase to hydrolyze the freed ATP, thus allowing a more accurate determination of the bacterial ATP which will subsequently be freed by acid extractant. The apyrase then is destroyed by adding a strong inorganic acid (e.g., nitric acid). When the acid is added, the bacterial cells are ruptured, freeing their ATP. The acid extractant then is diluted with distilled water.

At this point, the only remaining ATP in the suspension is bacterial. A firefly luciferase-luciferin mixture in soluble form and a small quantity of magnesium sulfate are added to the suspension. The luciferase is an enzyme which catalyzes the reaction of luciferin with ATP in the presence of magnesium ions. The reaction of the luciferin with the bacterial ATP produces light, the intensity of which is directly proportional to the amount of bacteria present. Light intensity is measured directly using a photometer. Amounts of chemically synthesized ATP are used for calibration. This procedure does not rely on growth and can be accomplished in less than one hour. It can detect the presence of as few as 1000 bacteria/ml from 10 ml of sample.

(continued overleaf)

Note:

Requests for further information may be directed to:
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Patent status:

This invention is owned by NASA and a patent application has been filed. Inquiries concerning non-exclusive or exclusive license for its commercial development should be addressed to:

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